PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P703PC00 - MNN/stc	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/DK 03/00585	International filing date *(day/month/year)* 10.09.2003	Priority date *(day/month/year)* 10.09.2002
International Patent Classification (IPC) or both national classification and IPC C12N15/62		
Applicant NATIMMUNE A/S et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
05.03.2004	29.12.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Heckl, K Telephone No. +49 89 2399-8430

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/DK 03/00585

I: Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-110 as originally filed

Sequence listings part of the description, Pages

1-237 as originally filed

Claims, Numbers

1-47 received on 20.12.2004 with letter of 20.12.2004

Drawings, Sheets

1/11-11/11 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 35-39

   because:

   ☒ the said international application, or the said claims Nos. 35-39 relate to the following subject matter which does not require an international preliminary examination (specify):

   see separate sheet

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	1-47
	No: Claims	
Inventive step (IS)	Yes: Claims	1-47
	No: Claims	
Industrial applicability (IA)	Yes: Claims	1-34,40-47
	No: Claims	

2. Citations and explanations

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1.
Claims 35-39 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

For the assessment of the present claims 35-39 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

The following documents are referred to in this written opinion:

D1: WO 02/06460 A (THIEL STEFFEN ;JENSENIUS JENS CHRISTIAN (DK)) 24 January 2002 (2002-01-24)

D2: WO 99/10001 A (TAKARA SHUZO CO ;KAWASAKI TOSHISUKE (JP)) 4 March 1999 (1999-03-04)

D3: WO 99/37676 A (FUSO PHARMACEUTICAL IND ;WAKAMIYA NOBUTAKA (JP)) 29 July 1999 (1999-07-29)

D4: WO 00/70043 A (JENSEN THOMAS VORUP ;THIEL STEFFEN (DK); JENSENIUS JENS CHRISTIAN) 23 November 2000 (2000-11-23)

D5: US-B-6 337 1931 (RONNING MICHAEL T ET AL) 8 January 2002 (2002-01-08)

D6: MATSUSHITA MISAO ET AL: "The role of ficolins in innate immunity." IMMUNOBIOLOGY. GERMANY SEP 2002, vol. 205, no. 4-5, September 2002 (2002-09), pages 490-497, XP002270462 ISSN: 0171-2985

D7: FUJITA TEIZO: "Evolution of the lectin-complement pathway and its role in innate immunity." NATURE REVIEWS. IMMUNOLOGY. ENGLAND MAY

2002, vol. 2, no. 5, May 2002 (2002-05), pages 346-353, XP002270463
ISSN: 1474-1733

D8: WO 01/40451 A (THIEL STEFFEN ;JENSENIUS JENS CHRISTIAN (DK)) 7 June 2001 (2001-06-07)

Novelty (Art.33(2) PCT):

The subject-matter of the claims is novel. In fact:

D1 discloses MASP-2 (mannin-binding lectin associated serine protease). MASP-2 is not a fusion protein which also has the features of the first and second polypeptide sequence according to claims 2 and 16/17.

D2 discloses MBL (mannan binding lectin) which shares 70% homology with SEQ.ID.NO.118 which represents the sequence of one embodiment falling into the scope of the claims. However, MBL is not a fusion protein which also has the feature of the first and second polypeptide sequences according to claims 2 and 16/17. In addition, D2 is totally silent about fusion proteins comprising MBL.

Similarly, D3, D4 and D5 relate to MBP/MBL.

D6 discusses the role of ficolins, MBL and MASP in complement activation. In fact, it is disclosed that MBL is closely related to ficolins in function and structure. Furthermore, MBL is associated with MASPs. When MBL-MASP binds to carbohydrates on the surface of microbes, MASPs acquire proteolytic activities and activate the complement components C4, C2 and C3. The importance of this so called MBLectin pathway in innate immunity of vertebrates is discussed, as well (see D6, Abstract, Introduction and Discussion).

D7 relates to the evolution of the lectin-complement pathway and its role in innate immunity.

D8 relates to recombinant MASP-3 and the use therefore in medicine.

Inventiveness (Art.33(3) PCT):

D6 is considered closest prior art. In its light, it was the problem underlying the claims to provide an alternative to the MBL-MASP complex and to apply this alternative in the treatment or prevention of infection.

As a solution, the present claims provide fusion proteins which should mimic the function of the naturally occurring ficolin/MBL complexed with MASPs.

In addition, the teaching of claim 1 relates to the principle of combining the function of a first polypeptide sequence which is capable of activating the lectin-complement pathway (such as MASP), and a second polypeptide sequence which is capable of associating with one or more carbohydrates and being derived from a collectin (such as MBL).

In the IPEA's opinion, this solution is not rendered obvious by any of the cited prior art. Therefore, it is considered inventive.

Claims

1. A fusion protein comprising
   i) A first polypeptide sequence derived from a lectin-complement pathway activating protein or a functional homologue thereof; and
   ii) A second polypeptide sequence derived from a collectin or a functional homologue thereof;
   wherein said complement activating protein is not a collectin.

2. The fusion protein according to claim 1, wherein said first polypeptide sequence is capable of activating the lectin-complement pathway.

3. The fusion protein according to claim 1, wherein said first polypeptide sequence is capable of associating with at least one MASP protein.

4. The fusion protein according to claim 1, wherein said first polypeptide sequence is capable of associating with a MASP protein selected from the group consisting of MASP-1, MASP-2 and MASP-3 or functional homologues or variants hereof.

5. The fusion protein according to claim 1, wherein the complement activating protein is a ficolin.

6. The fusion protein according to claim 5, wherein the ficolin is selected from the group consisting of L-ficolin, H-ficolin and M-ficolin.

7. The fusion protein according to claim 5, wherein the ficolin is L-ficolin.

8. The fusion protein according to any of claims 1 to 7, wherein said first polypeptide sequence comprises at least 10, such as at least 12, for example at least 15, such as at least 20, for example at least 25, such as at least 30, for example at least 35, such as at least 40, for example at least 50 consecutive amino acids of a complement activating protein or a sequence at least 70%, such as 80%, for example 90%, such as 95% identical thereto.

9. The fusion protein according to claim 1, wherein the first polypeptide sequence comprises the collagen-like domain of a ficolin or a functional homologue or variant thereof.

10. The fusion protein according to claim 1, wherein the first polypeptide sequence comprises the collagen-like domain of L-ficolin.

11. The fusion protein according to claim 1, wherein the first polypeptide sequence comprises the cysteine-rich region of a ficolin or a functional homologue thereof.

12. The fusion protein according to claim 1, wherein first polypeptide sequence comprises the cysteine-rich region of L-ficolin

13. The fusion protein according to claim 1, wherein the first polypeptide sequence comprises the cysteine-rich region and the collagen-like domain of a ficolin or a functional homologue or variant thereof.

14. The fusion protein according to claim 1, wherein first polypeptide sequence comprises the cysteine-rich region and the collagen-like domain of L-ficolin.

15. The fusion protein according to claim 1, wherein the first polypeptide sequence comprises amino acids 1-77 SEQ ID. NO 1.

16. The fusion protein according to claim 1, wherein said second polypeptide sequence is capable of associating with one or more carbohydrates.

17. The fusion protein according to claim 1, wherein the collectin is selected from the group consisting of MBL (mannose-binding lectin), SP-A (lung surfactant protein A), SP-D (lung surfactant protein D), BK (or BC, bovine conglutinin) and CL-43 (collectin-43).

18. The fusion protein according to claim 17, wherein the collectin is MBL.

19. The fusion protein according to any of claims 1 to 18, wherein said second polypeptide sequence comprises at least 10, such as at least 12, for example at

least 15, such as at least 20, for example at least 25, such as at least 30, for example at least 35, such as at least 40, for example at least 50 consecutive amino acids of a collectin or a sequence at least 70%, such as 80%, for example 90%, such as 95% identical thereto.

20. The fusion protein according to claim 1, wherein the second polypeptide sequence comprises the CRD domain of a collectin or a functional homologue or vaiant thereof.

21. The fusion protein according to claim 1, wherein the second polypeptide sequence comprises the CRD domain of MBL.

22. The fusion protein according to claim 1, wherein the second polypeptide sequence comprises the neck region of MBL.

23. The fusion protein according to claim 1, wherein the second polypeptide sequence comprises the collagen-like domain of MBL.

24. The fusion protein according to claim 1, wherein the second polypeptide sequence comprises the neck region and the CRD domain of MBL.

25. The fusion protein according to claim 1, wherein the second polypeptide sequence comprises the collagen-like domain, the neck region and the CRD domain of MBL.

26. The fusion protein according to claim 1, wherein the second polypeptide sequence comprises amino acids 80-228 SEQ ID. NO 2.

27. The fusion protein according to claim 1, wherein the fusion protein comprises the the cysteine-rich region and the collagen-like domain of L-ficolin and the CRD domain of MBL.

28. The fusion protein according to claim 1, wherein the fusion protein comprises the cysteine-rich region of L-ficolin and the collagen-like domain, the neck region and the CRD domain of MBL.

29. The fusion protein according to claim 1, wherein the fusion protein comprises the amino acid sequence as defined by SEQ ID. NO. 3, or a functional homologue thereof.

30. The fusion protein according to claim 1, wherein the fusion protein consists of the amino acid sequence as defined by SEQ ID. NO. 3.

31. An isolated nucleic acid comprising a nucleotide sequence encoding the fusion protein according to any of claims 1 to 30.

32. A vector comprising the nucleic acid sequence according to claim 31.

33. A cell comprising the vector according to claim 32.

34. The cell according to claim 33, wherein the cell is a mammalian cell.

35. The cell according to claim 33, wherein the cell is a non-mammalian cell.

36. A fusion protein according to any of claims 1 to 30 for use as a medicament.

37. A method of treatment of a clinical condition in an individual in need thereof comprising administering to said individual the fusion protein according to any of claims 1 to 30.

38. The method according to claim 37, wherein the clinical condition is an infection.

39. The method according to claim 37, wherein the individual is a human being.

40. The method according to claim 37, wherein the individual is a human being suffering from an increased risk of acquiring an infection.

41. The method according to claim 37, wherein the individual is a human being with subnormal serum MBL level.

42. The method according to claim 37, wherein the individual is a human being with normal serum MBL level.

43. Use of the fusion protein according to any of claims 1 to 30 for the preparation of a medicament for the treatment of a clinical condition in an individual in need thereof.

44. The use according to claim 43, wherein the clinical condition is an infection.

45. The use according to claim 43, wherein the individual is a human being.

46. The use according to claim 43, wherein the individual is a human being suffering from an increased risk of acquiring an infection.

47. The use according to claim 43, wherein the individual is a human being with sub-normal serum MBL level.

48. The use according to claim 43, wherein the individual is a human being with normal serum MBL level.

49. A medicament for the treatment or prevention of a clinical condition in an individual in need thereof, comprising the fusion protein according to any of claims 1 to 30.

50. The medicament according to claim 49, wherein the clinical condition is an infection.

51. The medicament according to claim 49, wherein the individual is a human being.